Experiment SH-84-2012

The effects of dietary sulfur source on ruminal hydrogen sulfide gas concentrations in feedlot lambs

Chloe Long

Department of Animal Sciences

Advisor: Dr. Steve Loerch

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INTRODUCTION

The demand for a bio-fuel option like ethanol has caused a substantial increase in the production of ethanol from corn. In fact, in 2002 only 20 million metric tons of corn was used for ethanol (DiConstanzo, 2007). Today, a calculated total of about 140 million metric tons of corn is used for ethanol production in the United States (Calculation 1; Carter et al., 2012; USDA-ERS, 2013; USDA-NASS, 2014). The increase in ethanol production from corn also increased production of its by-products, namely dried distillers grains plus solubles (DDGS; Klopfenstein et al., 2008). Livestock producers purchased and fed DDGS instead of corn, because of advantages in price due to competition with the ethanol industry during the early 2000s. Thus, DDGS became a popular feed ingredient for livestock.

DDGS is a bi-product of processing corn into ethanol, which takes place in five steps: milling, liquefaction, saccharification, fermentation, and distillation and recovery (Mosier et al., 2006). After the process is complete, sulfuric acid (H_2SO_4) is used to clean out fermentation tanks (Klopfenstein et al., 2008). In ruminants, increasing dietary inclusion of DDGS increases dietary sulfur (S) content (Klopfenstein et al., 2008) while also reducing rumen pH due to the residual H_2SO_4 in the fermentation tanks (Felix and Loerch, 2011).

DDGS contains between 0.5% and 1.2% dietary S. Cattle and sheep require 0.15% dietary S (NRC, 2000) and sulfur toxicity in these animals may take place above 0.4% dietary S (NRC, 2005). As a consequence of dietary S toxicity, risk of polioencephalomalacia (PEM) increases when dietary S is above 0.5% (Buckner et al., 2007). Sulfur-induced PEM involves the bacterial reduction of sulfate to sulfide (S^{2-}) and the protonation of S^{2-} to hydrogen sulfide gas (H_2S; Beauchamp et al., 1984) by sulfate reducing bacteria (SRB) in a pH dependent process. Elevated concentrations of H_2S in the rumen increase the incidence of S-induced PEM (Gould,

1998). We postulate that SRBs may require time to adapt to dietary S, and this may be important when transitioning cattle or sheep to DDGS-based diets that exceed the maximum tolerable level of S.

Previous research at The Ohio State University determined that it takes at least 28 days for SRB to reach maximum ability to reduce sulfide in the rumen and generate maximum concentrations of H_2S gas. Source of dietary S may affect this adaption process. However, data regarding the abrupt exposure of ruminants to different sources of dietary S and the effects on ruminal H_2S are lacking. We hypothesized that abrupt exposure to 0.42% added dietary S from DDGS and sulfuric acid will result in high concentrations of H_2S within the first 14 days of the experiment, whereas S from $NaSO_4$ will result in a delayed H_2S response with high concentrations not occurring until day 28 of the experiment. Therefore, the objectives of this study were to determine the effects dietary S source on H_2S concentration after abrupt dietary exposure to high concentrations of S.

MATERIALS AND METHODS

All animal procedures were approved by the Agricultural Animal Care and Use Committee of The Ohio State University and followed guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010.)

Animals and Diets

Sixty-nine, 5 to 6 month old Hampshire × Dorset ewe (n= 33) and wether (n=36) lambs, were blocked by either heavy or light body weight (BW=51.1 ± 0.4 kg) and sex (ewe or wether). Lambs were allotted within blocks to 23 pens consisting of 3 lambs per pen, 11 of which were pens of ewe lambs and 12 of which were pens of wether lambs. For 56 days prior to the abrupt

exposure to the experimental diets all lambs were fed a corn-based control diet that contained no supplemental dietary S (0.11% S on a DM basis).

The experimental diets were (Table 1) 1) 60% DDGS-based diet, 2) corn-based diet supplemented with 1.4% $NaSO_4$, and 3) corn-based diet treated with 2.1% of 9M H_2SO_4. Each of the three experimental dietary treatments were formulated to contain 0.4% S (NRC, 2005). The remainder of the diets consisted of soybean hulls, ground corn, and supplemental protein, minerals, and vitamins. The DDGS used in this study contained 0.68% S and was obtained from a single source (POET Biorefining; Marion, OH). The H_2SO_4 treated diet was made by weighing 150 kg of corn DM into a small ribbon mixer and treating each batch with 2.6% of 9M H_2SO_4. The remaining ingredients of the diet were added to the treated corn to create the final diet that was 2.1% of 9M H_2SO_4.

The pH of each of the dietary treatments was obtained by grinding 20g of each diet through a 2mm screen and adding 80mL of distilled H_2O and using a magnetic stir bar on a stir plate for 30 seconds and them recording the pH using a pH meter (Accument excel XL 25 dual channel pH/ion meter; Fisher Scientific).

The experiment was conducted at The Ohio State Agricultural Research and Development Center Sheep Center in Wooster, Ohio. The diets were fed in a complete pellet and offered ad libitum once daily at 0800. Prior to feed delivery, feed refusals were weighed and recorded daily. Lambs were monitored for abnormalities daily and one lamb was removed during the first week of the trial due to lameness.

Sampling and Analysis

Every other week feed samples were collected, composited, freeze dried (Freeze Dryer 8; Labconco, Kansas City, MO), and ground using a Wiley mill (1mm screen; Arthur H. Thomas,

Philadelphia, PA). All feeds were analyzed for DM by weighing a sample of the feed, placing it in a 100°C oven for 24 hours and weighing the dried sample and calculating the difference. The freeze dried samples were subjected to perchloric acid digestion and inductively coupled plasma atomic emission spectroscopy (ICP) for analysis of complete minerals (Method 965.03: AOAC, 1988). Additionally, feed samples were collected to analyze ADF and NDF (using Ankom Technology Method 5 and 6, respectively; Ankom^{200} Fiber Analyzer, Ankom Technology, Fairport, NY), CP (Method 930.15; AOAC, 1996), and fat (using ether extract method; Ankom Technology, Fairport, NY). The trial ended after the lambs were on feed for 27 days. Initial and final BW were the mean two consecutive weights taken at the beginning and end of the experiment and intermediate BW was measured on day 14.

Rumunocentesis

The effects of each of the dietary treatment in ruminal S metabolism were observed by collecting samples of rumen gas(via ruminocentesis) on all lambs at 6 hours post feeding on day 1, 14, 27, and analyzing H_2S concentration using a procedure adapted from Gould et al. (1997). In short, the skin in the left paralumbar fossa was shaved with surgical shears, scrubbed with 5% betadine, then rinsed with 70% ethanol, and numbed with local anesthetic. Ruminal gas was aspirated through the skin of the left paralumbar fossa at 6 hours post-feeding via puncture with a sterile 16-gauge, 3.81 centimeter hypodermic needle. The H_2S gas concentration was measured via H_2S precision gas detector tubes (No. 120SF, Sensidyne®, Ocala, FL) attached to a calibrated gas detection pump (Model AP-20S, Sensidyne®). At each sampling the same individual read the concentration of H_2S from the tube.

Statistical Analysis

The experimental design for this experiment was a complete randomized block design with 3 treatments. The data were analyzed using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). Repeated measures were used to analyze the effect of sampling time on ruminal H_2S using the covariate structure for unstructured data. The model included effects of time as a repeated measure and the time by treatment interaction. Pen was the experimental unit. Significance was declared at $P \leq 0.05$ and trends were discussed when $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Dry matter intake was 1.45 kg/d while the lambs were on the 14-day transition diet prior to the experimental treatments. After the diets were switched to the experimental treatments on d 1, lambs fed $NaSO_4$ treated corn (pH 6.18) had greater ($P < 0.01$) DMI than lambs fed DDGS (pH 4.57) or H_2SO_4 treated corn (pH 4.01) throughout the experiment. There was a similar response in ADG caused by increased the DMI. Lambs fed $NaSO_4$ also had greater ($P < 0.01$) ADG throughout the trial than those fed the other two diets, thus affecting feed efficiency (G:F). Lambs fed the $NaSO_4$ diet were the most efficient ($P < 0.01$). The increase in dietary pH was most likely the cause of the improvement in performance, and may have reduced the risk for metabolic acidosis. Felix et al. (2012a) reported a linear decrease in feedlot lamb performance with increasing dietary inclusion of DDGS which linearly reduced dietary pH where 60% DDGS diets were fed containing similar dietary S content. Thus suggesting that dietary pH has a more adverse effect on animal performance than diets containing high levels of dietary S alone.

H_2S concentrations of lambs fed $NaSO_4$ were below 200 mg/L on d 1, 14, and 27. Lambs fed 60% DDGS had H_2S concentrations under 200 mg/L on d 1, 2,200 on d 14, and 1,950 on d

27. Lambs fed H_2SO_4 had H_2S concentrations below 200 mg/L on d 1, 800 on d 14, and 600 on d 27. There was a time by S source interaction ($P < 0.01$). At the same dietary S inclusion, lambs fed the acidic sources of S (DDGS and H_2SO_4) had increased ruminal H_2S concentrations and decreased DMI and ADG when compared to lambs fed a non-acidic source of dietary S ($NaSO_4$). We hypothesized that lambs fed either the H_2SO_4 treated corn diet or DDGS would evoke the same ruminal H_2S responses because most of the dietary S in DDGS is believed to come from H_2SO_4 (Felix and Loerch, 2011). Yet, even though dietary S concentrations were the same in the DDGS and H_2SO_4 treatments, this was not the case. This led us to believe that dietary acidity contributes to an increased risk of S-induced PEM. According to Gould (1998) a lamb with a ruminal H_2S concentration of more than 2,000 mg/L is at risk for PEM. Consequently, lambs fed DDGS in this trial were at risk for PEM.

These data suggest that when DDGS-based diets are fed to lambs, dietary S concentration is not the only factor capable of reducing intake and growth. Therefore, other factors, along with dietary acidity and elevated dietary S concentrations, may be contributing to increased H_2S concentrations in feedlot lambs fed DDGS-based diets.

One assay that if measured, would have given us a different approach on which to draw conclusions is rumen pH. When ruminants are fed 60% DDGS the rumen pH declines very quickly in the first 3 hours after feeding and continues to remain low (~5.5) for up to 12 hours later (Felix et al., 2012b). If normal rumen pH is between 6.0 and 6.4, this drop in pH for extended amounts of time could affect the environment adversely. If we could have had the ability to measure rumen pH it might have given us a more accurate idea of the ways in which pH dependent processes (ie. SRB) in the rumen are affected by feeding acidic feeds like DDGS and if this has any effects on H_2S concentrations.

IMPLICATIONS

This experiment showed that dietary acidity plays a role in decreasing lamb performance and increasing the risk of S induced PEM. If the ethanol industry continues to grow and DDGS remains a common feed ingredient fed to livestock, further research to discover how to reduce the acidity of DDGS may help to reduce the risk of PEM. Also, more information on the effects of SRB on rumen microbiology when S containing feedstuffs are fed could be studied to build upon the findings of the current study.

TABLES AND GRAPHS

Table 1. Diet composition

	Dietary S Source		
Item, % DM Basis	DDGS[1]	H_2SO_4[2]	$NaSO_4$[3]
DDGS	60.00	0	0
Corn, ground	19.52	70.686	71.386
Soybean hulls	15.00	15.00	15.00
Soybean meal	0	7.30	7.30
Urea	0	0.50	0.50
Limestone	2.20	1.15	1.15
Trace mineral salt[4]	0.50	0.50	0.50
Vitamin A, 30,000 IU/g	0.010	0.010	0.010
Vitamin D, 3,000 IU/g	0.010	0.010	0.010
Vitamin E, 44 IU/g	0.052	0.052	0.052
Selenium, 201 mg/g	0.156	0.156	0.156
Bovatec[5]	0.015	0.015	0.015
Ammonium chloride	0.521	0.521	0.521
Animal/vegetable fat[6]	2.00	2.00	2.00
9 M H_2SO_4	0	2.10	0
$NaSO_4$	0	0	1.40
Analyzed Composition			
NDF, %	27.8	15.3	17.5
ADF, %	14.0	11.1	10.8
CP, %	20.5	14.2	13.5
EE[7], %	10.5	3.9	6.4
Ca, %	0.96	0.61	0.65
P, %	0.52	0.24	0.23
S, %	0.38	0.38	0.33
Diet pH	4.57	6.18	4.01

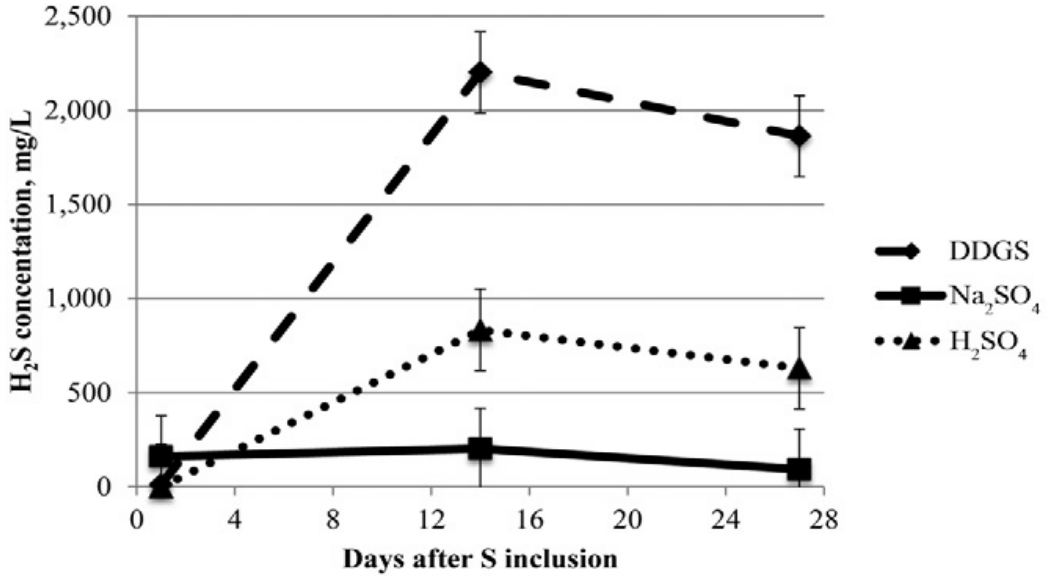
[1]Dried distillers grains with solubles; fed at 60% of diet DM
[2]Corn-based diet treated with 2.1% 9 M H_2SO_4
[3]Corn-based diet supplemented with 1.4% $NaSO_4$
[4]Included: 95% NaCl; 0.35% Zn, as ZnO; 0.28% Mn, as MnO_2; 0.175% Fe, as $FeCO_3$; 0.040% Cu, as Cu_2O; 0.007% I, as $Ca_5(IO_6)_2$; 0.007% Co, as $CoCO_3$
[5]Fed to provide 19.1 mg lasalocid/kg of diet DM (Bovatec®; Alpharma Animal Health, Bridgewater, NJ)
[6]Added to improve pellet quality
[7]EE= ether extractable fat

Table 2. Effects of S source on lamb performance

Item	Dietary S Source			SEM	*P*-value
	DDGS[1]	H_2SO_4[2]	Na_2SO_4[3]		
Animals (pens)	20 (7)	24 (8)	24 (8)		
BW, kg					
d 1	51.0	50.8	51.6	0.4	0.36
d 14	53.9[b]	54.2[b]	56.8[a]	0.5	<0.01
d 17	57.1[b]	56.7[b]	61.4[a]	0.6	<0.01
ADG, g					
d 1-14	205[b]	244[b]	373[a]	22	<0.01
d 15-27	245[b]	190[b]	351[a]	32	<0.01
d 1-27	224[b]	219[b]	363[a]	15	<0.01
DMI, kg/d					
d -14 to 0	1.46	1.44	1.46	0.03	0.88
d 1-14	1.37[b]	1.33[b]	1.57[a]	0.03	<0.01
d 15-27	1.48[b]	1.40[b]	1.72[a]	0.05	<0.01
d 1-27	1.42[b]	1.37[b]	1.64[a]	0.03	<0.01
G:F, kg/kg					
d 1-14	0.150[b]	0.184[b]	0.237[a]	0.016	<0.01
d 15-27	0.164[ab]	0.135[b]	0.203[a]	0.018	0.03
d 1-27	0.158[b]	0.160[b]	0.220[a]	0.008	<0.01

[a,b] Means in the same row without a common superscript differ ($P \leq 0.05$)
[1]DDGS = dried distillers grains with solubles; fed at 60% of diet DM.
[2]Corn based diet treated with 2.1% 9 *M* H2SO4.
[3]Corn-based diet supplemented with 1.4% Na2SO4.

Figure 1. Effects of S source on ruminal H_2S concentrations



Source of S was either dried distillers grains with solubles (DDGS; ♦), sodium sulfate treated corn (Na_2SO_4; ■), or sulfuric acid treated corn (H_2SO_4; ▲). Main effects of S source ($P < 0.01$) and time ($P < 0.01$) were detected. There was a time × S source interaction ($P < 0.01$). Error bars are associated with the time × S source interaction (SEM = 215).

Calculation 1. Corn Used for Ethanol Estimate

- Average production of corn per acre in 2014 (USDA-NASS, 2014) = 174.2 bu/acre
- Corn planted in the U.S. (USDA-ERS, 2013) = 80 mil acres U.S. corn planted/year
- Percentage of corn production that goes towards ethanol (Carter & Miller, 2012) = 40%

174.2 bu/acre × 80 million acres of corn in the U.S. per year = 1.39×10^{10} bu corn

1.39×10^{10} bu corn in US × 40% sold to ethanol production = 5.57×10^{9} bu corn towards ethanol

5.57×10^{9} bu corn towards ethanol × 56lbs/bu × 0.45kg/lb × 0.001 metric ton/kg = 140,474,880 metric tons of corn used for ethanol in the U.S. per year

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